UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
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SAM - 115

9 CFR 113.146	Revised June, 1985	Bovine Virus Diarrhea
Standard Requirement	Supersedes June 14, 1971	Agent

SUPPLEMENTAL ASSAY METHOD

FOR

TITRATION OF BOVINE VIRUS DIARRHEA

NEUTRALIZING ANTIBODY

(Constant Serum——Varying Virus Method)

A. SUMMARY

This is an in vitro assay method which uses a cell culture system for determining the antibody titer of serum against Bovine Virus Diarrhea (BVD) virus.

B. MATERIALS

1. Cell Cultures. Tissue culture chamber/slides (8 chamber/slide), containing monolayers of bovine embryonic kidney (BEK) cells, are used to titrate serum antibody. Only cells found to be free of extraneous agents are used (9 CFR 113.51 or 113.52).

2. Growth Medium. Minimum Essential Medium (MEM), Appendix (1) is used for growth of cells.

3. Diluent. MEM without serum is used for dilutions of indicator virus.

4. Indicator Virus. A Veterinary Biologics BVD reference virus is used.

5. Conjugate. Veterinary Biologics fluorescein conjugated BVD specific-immune bovine serum is used to stain the cell monolayer.

C. METHOD

1. All sera are heat treated at 56C for 30 minutes.
2. For each serum, equal amounts are placed into a series of tubes.
3. For a negative control, the same amounts of medium are placed into a series of tubes.
4. Serial 10-fold dilutions of the indicator virus are made in tubes containing diluent (Appendix 1, without serum). The virus dilutions

used for the test is dependent upon the known titer of the virus (usually use dilutions from 2 logs lower and one log higher than the virus titer).

5. Starting with the higest dilution of virus each dilution is placed into the corresponding tube containing an equal amount of serum or diluent.
6. The contents of each tube is mixed and allowed to incubate at room temperature for 30-45 minutes.
7. One-tenth amounts of each serum-virus mixture are placed into Lab-Tek chamber slides containing a monolayer of embryonic bovine kidney cells.
8. The inoculated cells ae incubated at 35-37 C in an atmosphere of 5% carbon dioxide and high humidity for 3 to 5 days.
9. The cells are examined for cytopathology and/or can be processed for BVD specific fluorescence staining as follows:
   a. The plastic lids and chamber walls are removed from the slides.
   b. The cells are quickly rinsed in phosphate buffered saline (appendix 2), then in demineralized water, and air dried.
   c. The cells are fixed in acetone for 15 minutes, then allowed to dry.
   d. The slides are covered with conjugated BVD specific-immune serum and held in a high humidity 37 C incubator for 30 minutes.
   e. Excess conjugate is washed from the cells in a gently circulating PBS bath for 10 minutes, then rinsed in demineralized water and air dried.

10. The cells are examined fo cytopathology or fluorescence, and the serum neutralization index can be determined.

APPENDIX

1. Minimum Essential Medium (MEM)

MEM (Eagle) with Earle's salts q.s. ad	100.0%
Edamin	0.5%
L-Glutamine	1.0%
Antibiotics - Gentamicin	50.0 mcg per ml
Penicillin	100.0 units per ml
Streptomycin	100.0 mcg per ml
Amphotericin B	2.5mcg per ml
Fetal Bovine Serum	10.0%

2. Phosphate Buffered Saline (PBS-Dulbecco)

NaCl	0.8 %
KCl	0.02 %
Na_2HPO_4	0.115%
KH_2PO_4	0.02 %
$CaCl_2$ (anhy)	0.01 %
$Mgcl_2$ $6H_2O$	0.01 %
Distilled H_2O q.s. ad	100.0 %